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(54) Title: HYBRID PROTEINS WHICH FORM HETERODIMERS

(57) Abstract

A hybrid protein includes two coexpressed amino acid sequences forming a dimer. Each sequence contains the binding portion of a receptor, such as TBP1 or TBP2, or a ligand, such as IL-6, IFN-β and TPO, linked to a subunit of a heterodimeric proteinaceous hormone, such as hCG. Each coexpressed sequence contains a corresponding hormone subunit so as to form a heterodimer upon expression. Corresponding DNA molecules, expression vectors and host cells are also disclosed as are pharmaceutical compositions and a method of producing such proteins.

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HYBRID PROTEINS WHICH FROM HETERODIMERS

FIELD OF THE INVENTION

The present invention relates to a hybrid protein comprising two coexpressed amino acid sequences forming a dimer, each comprising:

a) at least one amino acid sequence selected from a homomeric receptor, a chain of a heteromeric receptor, a ligand, and fragments thereof; and

b) a subunit of a heterodimeric proteinaceous hormone or fragments thereof; in which (a) and (b) are bonded directly or through a peptide linker, and, in each couple, the two subunits (b) are different and capable of aggregating to form a dimer complex.

BACKGROUND OF THE INVENTION

Protein-protein interactions are essential to the normal physiological functions of cells and multicellular organisms. Many proteins in nature exhibit novel or optimal functions when complexed with one or more other protein chains. This is illustrated by various ligand-receptor combinations that contribute to regulation of cellular activity. Certain ligands, such as tumor necrosis factor α (TNFα), TNFβ, or human chorionic gonadotropin (hCG), occur as multi-subunit complexes. Some of these complexes contain multiple copies of the same subunit. TNFα and TNFβ (collectively referred to hereafter as TNF) are homotrimers formed by three identical subunits (1-4). Other ligands are composed of non-identical subunits. For example, hCG is a heterodimer (5-7). Receptors may also occur or function as multi-chain complexes. For example, receptors for TNF transduce a signal after being aggregated to form dimers (8,9). Ligands to these receptors promote aggregation of two or three receptor chains, thereby affording a mechanism of receptor activation. For example, TNF-mediated aggregation activates TNF receptors (10-12).

The modulation of protein-protein interactions can be a useful mechanism for therapeutic intervention in various diseases and pathologies. Soluble binding proteins, that can

interact with ligands, can potentially sequester the ligand away from the receptor, thereby reducing the activation of that particular receptor pathway. Alternatively, sequestration of the ligand may delay its elimination or degradation, thereby increasing its duration of effect, and perhaps its apparent activity *in vivo*. In the case of TNF, soluble TNF receptors have been primarily associated with inhibition of TNF activity (13-17).

Soluble binding proteins may be useful for treating human diseases. For example, soluble TNF receptors have been shown to have efficacy in animal models of arthritis (18,19).

Since TNF has three binding sites for its receptor (10-12), and dimerization of the cell surface receptor is sufficient for bioactivity (8,9), it is likely that binding of a single soluble receptor to TNF will leave open the possibility that this 1:3 complex of soluble receptor:TNF (trimer) can still bind and activate a pair of cell surface TNF receptors. To achieve an inhibitory effect, it would be expected that two of the receptor binding sites on the TNF trimer must be occupied or blocked by the soluble binding protein. Alternatively, the binding protein could block proper orientation of TNF at the cell surface.

Generally speaking, the need was felt of synthesizing proteins that contain two receptor (or ligands) chains, as dimeric hybrid protein. See Wallach et al., U.S. patent 5,478,925.

The primary strategy employed for generating dimeric or multimeric hybrid proteins, containing binding domains from extracellular receptors, has been to fuse these proteins to the constant regions of an antibody heavy chain.

This strategy led, for example, to the construction of CD4 immunoadhesins (20). These are hybrid molecules consisting of the first two (or all four) immunoglobulin-like domains of CD4 fused to the constant region of antibody heavy and light chains. This strategy for creating hybrid molecules was adapted to the receptors for TNF (10,16,21) and led to the generation of constructs with higher *in vitro* activity than the monomeric soluble binding proteins.

It is widely held that the higher *in vitro* potency of the dimeric fusion proteins should translate into higher *in vivo* activity. One study does support this, revealing an at least 50-fold higher activity for a p75(TBP2)-Ig fusion protein in protecting mice from the consequences of intravenous LPS injection (16).

However, despite the widespread utilization of immunoglobulin fusion proteins, this strategy has several drawbacks. One is that certain immunoglobulin Fc domains participate in effector functions of the immune system. These functions may be undesirable in a particular therapeutic setting (22).

A second limitation pertains to the special cases where it is desirable to produce heteromeric fusion proteins, for example soluble analogs of the heteromeric IL-6 or type I interferon receptors. Although there are numerous methods for producing bifunctional antibodies (e.g., by co-transfection or hybridoma fusions), the efficiency of synthesis is greatly compromised by the mixture of homodimers and heterodimers that typically results (23). Recently there have been several reports describing the use of leucine zipper motifs to guide assembly of heterodimers (24-26). This appears to be a promising approach for research purposes, but the non-native or intracellular sequences employed may not be suitable for chronic applications in the clinic due to antigenicity. The efficiency of assembly and stability post assembly may also be limitations.

On the other hand, in the particular case of TNF receptors, certain modifications to the p55 TNF receptor have been found to facilitate homodimerization and signaling in the absence of ligand (27,28). It has been found that a cytoplasmic region of the receptor, termed the "death domain," can act as a homodimerization motif (28,30). As an alternative to an immunoglobulin hybrid protein, fusion of the extracellular domain of the TNF receptor to its cytoplasmic death domain could conceivably result in a secreted protein which can dimerize in the absence of TNF. Such fusion proteins

have been disclosed and claimed in the International Patent Application WO 95/31544.

A third further strategy employed for generating dimers of soluble TNF receptors has been to chemically cross-link the monomeric proteins with polyethylene glycol (31).

SUMMARY OF THE INVENTION

An alternative for obtaining such dimeric proteins, offering some important advantages, is the one of the present invention and consists in using a natural heterodimeric scaffold corresponding to a circulating non-immunoglobulin protein with a long half-life. A preferred example is hCG, a protein that is secreted well, has good stability, and has a long half-life (32-33). Given hCG's prominent role as a marker of pregnancy, many reagents have been developed to quantitate and study the protein *in vitro* and *in vivo* . In addition, hCG has been extensively studied using mutagenesis, and it is known that small deletions to the protein, such as removal of five residues at the extreme carboxyl-terminus of the α subunit, can effectively eliminate its biological activity while preserving its capability to form heterodimer (34,35). Small insertions, of up to 30 amino acids, have been shown to be tolerated at the amino- and carboxyl-termini of the α subunit (36), while fusion of the α subunit to the carboxyl terminus of the β subunit also had little effect on heterodimer formation (37).

An analog of hCG in which an immunoglobulin Fc domain was fused to the C-terminus of hCG β subunit has also been reported; however, this construct was not secreted and no effort was made to combine it with an α subunit (38).

Therefore, the main object of the present invention is a hybrid protein comprising two coexpressed amino acid sequences forming a dimer, each comprising:

a) at least one amino acid sequence selected among a homomeric receptor, a chain of a heteromeric receptor, a ligand, and fragments thereof; and

b) a subunit of a heterodimeric proteinaceous hormone, or fragments thereof; in which (a) and (b) are bonded

directly or through a peptide linker, and in each couple the two subunits (b) are different and capable of aggregating forming a dimer complex.

According to the present invention, the linker may be enzymatically cleavable.

Sequence (a) is preferably selected among: the extracellular domain of the TNF Receptor 1 (55 kDa, also called TBP1), the extracellular domain of the TNF Receptor 2 (75 kDa, also called TBP2), or fragments thereof still containing the ligand binding domain; the extracellular domains of the IL-6 receptors (also called gp80 and gp130); the extracellular domain of the IFN α/β receptor or IFN γ receptor; a gonadotropin receptor or its extracellular fragments; antibody light chains, or fragments thereof, optionally associated with the respective heavy chains; antibody heavy chains, or fragments thereof, optionally associated with the respective light chains; antibody Fab domains; or ligand proteins, such as cytokines, growth factors or hormones other than gonadotropins, specific examples of which include IL-6, IFN-β, TPO, or fragments thereof.

Sequence (b) is preferably selected among a hCG, FSH, LH, TSH, inhibin subunit, or fragments thereof.

Modifications to the proteins, such as chemical or protease cleavage of the protein backbone, or chemical or enzymatic modification of certain amino acid side chains, can be used to render the components of the hybrid protein of the invention inactive. This restriction of activity may also be accomplished through the use of recombinant DNA techniques to alter the coding sequence for the hybrid protein in a way that results directly in the restriction of activity to one component, or that renders the protein more amenable to subsequent chemical or enzymatic modification.

The above hybrid proteins will result in monofunctional, bifunctional or multifunctional molecules, depending on the amino acid sequences (a) that are combined with (b). In each couple, (a) can be linked to the amino termini or to the carboxy termini of (b), or to both.

A monoclonal hybrid protein of the present invention can, for instance, comprise the extracellular domain of a gonadotropin receptor linked to one of the corresponding receptor-binding gonadotropin subunits. According to such an embodiment, the hybrid protein of the invention can be a molecule in which, for example, the FSH receptor extracellular domain is linked to FSH to increase plasma half-life and improve biological activity.

This preparation can be employed to induce follicular maturation in assisted reproduction methods, such as ovulation induction or *in vitro* fertilisation, and to serve as a means to dramatically amplify the biological activity of the hormone essential for the success of the process, thus reducing the requirement for both the hormone itself and the number of injections to achieve ovulation.

The FSH receptor and the production of the extracellular domain of the human FSH receptor have been described respectively in WO 92/16620 and WO 96/38575.

According to a particular embodiment, the extracellular domain of the FSH receptor (ECD) can be fused in frame with a peptide linker that contains the thrombin recognition/cleavage site (29) and represents a "tethered" arm. The peptide linker links the extracellular domain of FSH with a FSH subunit. This will allow for removal of the extracellular domain of the FSH receptor by cleavage at the thrombin cleavage site as the molecule comes in contact with thrombin in the systemic circulation.

In another embodiment, instead of the thrombin cleavage site, an enzyme recognition site for an enzyme that is found in greatest abundance in the ovary is used. In this way, as the ECD-FSH molecule travels to the ovary, it will be exposed to enzymes found in the highest concentrations in that tissue and the ECD will be removed so that the FSH can interact with the membrane bound receptor.

In yet another embodiment, instead of an enzyme recognition site, a flexible hinge region is cloned between ECD and FSH so that the ECD will not be enzymatically removed from the hormone. In this way, when the ECD-FSH molecule arrives at

the ovary, a competition will be established between the hinge-attached ECD and the ECD of the FSH receptor found on the ovarian cell membrane.

In a further preferred embodiment of the invention, the hybrid protein consists of the aggregation between a couple of aa sequences, one of which contains TBP1 (or the fragments from aa 20 to aa 161 or to aa 190) as (a) and the α subunit of hCG as (b), and the other contains always TBP1 (or the same fragments as above) as (a) and the β subunit of hCG, or fragments thereof, as (b). According to this embodiment, depending on the particular sequence that is chosen as (b) (the entire β subunit of hCG, or fragments or modifications thereof), the resulting hybrid protein will have one activity (only that of TBP1) or a combination of activities (that of TBP1 with that of hCG). In this latter case the hybrid protein can be used, for example, in the combined treatment of Kaposi's sarcoma and metabolic wasting in AIDS.

In a further embodiment of the invention, one or more covalent bonds between the two subunits (b) are added to enhance the stability of the resulting hybrid protein. This can be done, e.g., by adding one or more non-native interchain disulfide bonds. The sites for these cross-links can be deduced from the known structures of the heterodimeric hormones. For example, a suitable site in hCG could be to place cysteine residues at α subunit residue Lys45 and β subunit residue Glu21, replacing a salt bridge (non-covalent bond) with a disufide bond (covalent bond). Another object of the present invention are PEGylated or other chemically modified forms of the hybrid proteins.

A further object of the present invention is a DNA molecule comprising the DNA sequence coding for the above hybrid protein, as well as nucleotide sequences substantially the same. "Nucleotide sequences substantially the same" includes all other nucleic acid sequences which, by virtue of the degeneracy of the genetic code, also code for the given amino acid sequence.

For the production of the hybrid protein of the invention, the DNA sequence (a) is obtained from existing

clones, as is (b). The DNA sequence coding for the desired sequence (a) is ligated with the DNA sequence coding for the desired sequence (b). Two of these fused products are inserted and ligated into a suitable plasmid or each into a different plasmid. Once formed, the expression vector, or the two expression vectors, is introduced into a suitable host cell, which then expresses the vector(s) to yield the hybrid protein of the invention as defined above.

The preferred method for preparing the hybrid of the invention is by way of PCR technology using oligonucleotides specific for the desired sequences to be copied from the clones encoding sequences (a) and (b).

Expression of any of the recombinant proteins of the invention as mentioned herein can be effected in eukaryotic cells (e.g., yeasts, insect or mammalian cells) or prokaryotic cells, using the appropriate expression vectors. Any method known in the art can be employed.

For example the DNA molecules coding for the proteins obtained by any of the above methods are inserted into appropriately constructed expression vectors by techniques well known in the art (see Sambrook et al, 1989). Double stranded cDNA is linked to plasmid vectors by homopolymeric tailing or by restriction linking involving the use of synthetic DNA linkers or blunt-ended ligation techniques: DNA ligases are used to ligate the DNA molecules and undesirable joining is avoided by treatment with alkaline phosphatase.

In order to be capable of expressing the desired protein, an expression vector should comprise also specific nucleotide sequences containing transcriptional and translational regulatory information linked to the DNA coding the desired protein in such a way as to permit gene expression and production of the protein. First in order for the gene to be transcribed, it must be preceded by a promoter recognizable by RNA polymerase, to which the polymerase binds and thus initiates the transcription process. There are a variety of such promoters in use, which work with different efficiencies (strong and weak promoters).

For eukaryotic hosts, different transcriptional and translational regulatory sequences may be employed, depending on the nature of the host. They may be derived form viral sources, such as adenovirus, bovine papilloma virus, Simian virus or the like, where the regulatory signals are associated with a particular gene which has a high level of expression. Examples are the TK promoter of the Herpes virus, the SV40 early promoter, the yeast gal4 gene promoter, etc. Transcriptional initiation regulatory signals may be selected which allow for repression and activation, so that expression of the genes can be modulated.

The DNA molecule comprising the nucleotide sequence coding for the hybrid protein of the invention is inserted into a vector(s), having the operably linked transcriptional and translational regulatory signals, which is capable of integrating the desired gene sequences into the host cell. The cells which have been stably transformed by the introduced DNA can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may also provide for phototrophy to a auxotropic host, biocide resistance, e.g., antibiotics, or heavy metals such as copper, or the like. The selectable marker gene can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of proteins of the invention.

Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Once the vector(s) or DNA sequence containing the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means: transformation,

transfection, conjugation, protoplast fusion, electroporation, calcium phosphate-precipitation, direct microinjection, etc.

Host cells may be either prokaryotic or eukaryotic. Preferred are eukaryotic hosts, e.g., mammalian cells, such as human, monkey, mouse, and Chinese hamster ovary (CHO) cells, because they provide post-translational modifications to protein molecules, including correct folding or glycosylation at correct sites. Also, yeast cells can carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene products and secretes peptides bearing leader sequences (i.e., pre-peptides).

After the introduction of the vector(s), the host cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene sequence(s) results in the production of the desired proteins.

Purification of the recombinant proteins is carried out by any one of the methods known for this purpose, i.e., any conventional procedure involving extraction, precipitation, chromatography, electrophoresis, or the like. A further purification procedure that may be used in preference for purifying the protein of the invention is affinity chromatography using monoclonal antibodies which bind the target protein and which are produced and immobilized on a gel matrix contained within a column. Impure preparations containing the recombinant protein are passed through the column. The protein will be bound to the column by the specific antibody while the impurities will pass through. After washing, the protein is eluted from the gel by a change in pH or ionic strength.

The term "hybrid protein", as used herein, generically refers to a protein which contains two or more different proteins or fragments thereof.

As used herein, "fusion protein" refers to a hybrid protein, which consists of two or more proteins, or fragments thereof, linked together covalently.

The term "aggregation", as used herein, means the formation of strong specific non-covalent interactions between two polypeptide chains forming a complex, such as those existing between the α and β subunit of a heterodimeric hormone (such as FSH, LH, hCG or TSH).

The terms "ligand" or "ligand protein", as used herein, refer to a molecule, other than an antibody or an immunoglobulin, capable of being bound by the ligand-binding domain of a receptor; such molecule may occur in nature, or may be chemically modified or chemically synthesised.

The term "ligand-binding domain", as used herein, refers to a portion of the receptor that is involved in binding a ligand and is generally a portion or essentially all of the extracellular domain.

The term "receptor", as used herein, refers to a membrane protein, whose binding with the respective ligand triggers secondary cellular responses that result in the activation or inhibition of intracellular process.

In a further aspect, the present invention provides the use of the hybrid protein as a medicament. The medicament is preferably presented in the form of a pharmaceutical composition comprising the protein of the invention together with one or more pharmaceutically acceptable carriers and/or excipients. Such pharmaceutical compositions represent yet a further aspect of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be better understood by reference to the appended drawings, in which:

Figures 1(a) and 1(b) show the TBP(20-161)-hCGα and TBP(20-161)-hCGβ constructs, respectively, and the corresponding sequences (SEQ ID NOS:1-4).

Figures 2(a) and 2(b) show the TBP(20-190)-hCGα and TBP(20-190)-hCGβ constructs, respectively, and the corresponding sequences (SEQ ID NOS:5-8).

Figure 3 is a schematic summary of the constructs of Figures 1 and 2 showing p55 TNFR1, TBP1 and TBP1 fusion contructs. The linker sequences shown on the last two lines are SEQ ID NO:9 (Ala-Gly-Ala-Ala-Pro-Gly) and SEQ ID NO:10 (Ala-Gly-Ala-Gly).

Figure 4 is a graph illustrating the dose dependent protective effect of CHO cell expressed TBP-hCG(20-190) on TNFα-induced cytotoxicity on BT-20 cells and various controls.

Figure 5 is a graph illustrating the dose dependent protective effect of COS cell expressed TBP-hCG(20-190) on TNFα-induced cytotoxicity on BT-20 cells and various controls.

Figure 6 is a graph illustrating the dose dependent protective effect of affinity purified CHO cell expressed TBP-hCG(20-161) on TNFα-induced cytotoxicity on BT-20 cells and various controls.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention will now be described by means of the following Examples, which should not be construed as in any way limiting the present invention.

EXAMPLES

Materials and Methods

Cell lines used in this study were obtained from the American Type Culture Collection (ATCC), Rockville, Maryland, unless otherwise specified. The CHO-DUKX cell line was obtained from L. Chasin at Columbia University through D. Houseman at MIT (39). The CHO-DUKX cells, which lack a functional gene for dihydrofolate reductase, were routinely maintained in complete α-plus Modified Eagles Medium (α(+)MEM) supplemented with 10% fetal bovine serum (FBS). The COS-7 cells were routinely maintained in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% FBS. Unless specified otherwise, cells were split to maintain them in log phase of growth, and culture reagents were obtained from GIBCO (Grand Island, New York).

1. Assembly of the genetic constructs encoding the <u>hybrid proteins</u>

The numbering assignments for the p55 TNF receptor are based on the cloning paper from Wallach (40), while the numbering assignments for the hCG subunits are based on the numbering assignments from the Fiddes cloning papers (41,42). The designation TBP, or TNF binding protein, refers to the extracellular domain portions of the TNF receptors capable of binding TNF. In these Examples, the DNA constructs will be named as TBP-hybrid proteins, with the partner and region of TBP indicated in the construct nomenclature. All of the TBP-hCG constructs contain the human growth hormone (hGH) signal peptide in place of the native p55 signal sequence. In addition, the hGH signal peptide has been placed so that it immediately precedes TBP residue Asp20, which is anticipated to make this the first residue in the mature, secreted protein. These modifications are not essential to the basic concept of using hCG as a partner of the hybrid protein.

The DNAs encoding the hybrid proteins were constructed using PCR methodology (43).

a. <u>TBP1(20-161)-hCG</u>

The initial TBP-hCG construct was engineered to contain the ligand binding domain from the extracellular region of the p55 TNF receptor (from Asp20 inclusive of residue Cys161) fused though a short linker to the hCG α and β subunits (starting at residues αCys7 or βPro7, respectively). This construct, hereafter referred to as TBP1(20-161)-hCG, is a heterodimer of two modified hCG subunits, TBP1(20-161)-hCGα and TBP1(20-161)-hCGβ.

The oligodeoxynucleotide primers used for the TBP1(20-161)-hCGα construct were:

primer 1($\alpha\beta$) TTT TCT CGA GAT GGC TAC AGG TAA GCG CCC (SEQ ID NO:11)

primer 2(α) ACC TGG GGC AGC ACC GGC ACA GGA GAC ACA CTC GTT TTC (SEQ ID NO:12)

primer 3(α) TGT GCC GGT GCT GCC CCA GGT TGC CCA GAA TGC ACG CTA CAG (SEQ ID NO:13)

primer 4 (α) TTT TGG ATC CTT AAG ATT TGT GAT AAT AAC AAG TAC (SEQ ID NO:14)

These and all of the other primers described in these Examples were synthesized on an Applied Biosystems Model 392 DNA synthesis machine (ABI, Foster City, California), using phosphoramidite chemistry.

Since both of the TBP-hCG subunit constructs have the same 5'-end (i.e., the 5'-end of the hGH/TBP construct), primer 1($\alpha\beta$) was used for both TBP-hCG subunit constructs. The other primers used for the TBP1(20-161)-hCGβ construct were:

primer 2(β) CCG TGG ACC AGC ACC AGC ACA GGA GAC ACA CTC GTT TTC (SEQ ID NO:15)

primer 3(β) TGT GCT GGT GCT GGT CCA CGG TGC CGC CCC ATC AAT (SEQ ID NO:16)

primer 4(β) TTT TGG ATC CTT ATT GTG GGA GGA TCG GGG TG (SEQ ID NO:17)

Primers 2(α) and 3(α) are reverse complements, and cover both the 3'-end of the coding region for the p55 extracellular domain, and the 5'-end of the hCG α subunit. Similarly, primers 2(β) and 3(β) are also reverse complements, and cover both the 3'-end of the coding region for the p55 extracellular domain, and the 5'-end of the hCG β subunit.

Two PCR reactions were run for each of the two TBP-hCG subunit constructs. The first used primers 1($\alpha\beta$) and 2 (α or β), and used as the template a plasmid encoding soluble p55 residues 20-180 preceded by the hGH signal peptide (plasmid pCMVhGHspcDNA.pA4). The second used primers 3 (α or β) and 4 (α or β), and used as the template either plasmid pSVL-hCGα or pSVL-hCGβ (44). The PCR was performed using Vent (TM) polymerase from New England Biolabs (Beverly, Massachusetts) in accordance with the manufacturer's recommendations, using for each reaction 25 cycles and the following conditions:

100 μg of template DNA
1 μg of each primer
2U of Vent(TM) polymerase (New England Biolabs)
denaturation at 99°C for 30 seconds
annealing at: 59°C for 30 seconds for primers 1($\alpha\beta$) and 2(α)

59°C for 30 seconds for primers 3(α) and 4(α)
57°C for 30 seconds for primers 1($\alpha\beta$) and 2(β)
63°C for 30 seconds for primers 3(β) and 4(β)
extension at 75°C for 75 seconds.

The PCR products were confirmed to be the expected size by electrophoresis in a 2% agarose gel and ethidium bromide staining. The fragments were then purified by passage over a Wizard column (Promega) in accordance with the column manufacturer's recommendations.

The final coding sequence for TBP1(20-161)-hCGα was assembled by fusion PCR using primer 1($\alpha\beta$) and primer 4(α), and using as template the purified products from the p55 and hCG α fragments obtained from the first PCR reactions. First the two templates, which due to the overlap between primers 2(α) and 3(α) could be denatured and annealed together, were passed through 10 cycles of PCR in the absence of any added primers. The conditions for these cycles were essentially the same as those used earlier, except that the annealing was done at 67°C and the extension was performed for 2 minutes. At the end of these 10 cycles, primers 1($\alpha\beta$) and 4(α) were added, and another 10 cycles were performed. The conditions for this final set of reactions was the same as used earlier, except that an annealing temperature of 59°C was used, and the extension was performed for 75 seconds.

Analysis of the products of this reaction by electrophoresis in a 1% agarose gel confirmed that the expected fragment of about 1100bp was obtained. The reaction was passed over a Wizard column to purify the fragment, which was then digested with XbaI and BamHI and re-purified in a 0.7% low-melting point agarose gel. The purified fragment was subcloned into plasmid pSVL (Pharmacia), which had first been digested with XbaI and BamHI and gel purified on a 0.8% low-melting point agarose gel. Following ligation with T4 ligase, the mixture was used to transform AG1 *E. coli* and then plated onto LB/ampicillin plates for overnight culture at 37°C. Plasmid DNAs from ampicillin-resistant colonies were analyzed by digestion with XhoI and BamHI to confirm the presence of the insert (which is excised in this digest). Six clones were

found to contain inserts, and one (clone 7) was selected for further advancement and designated pSVLTBPhCGα (containing TBP1(20-161)-hCGα). Dideoxy DNA sequencing (using Sequenase™, U.S. Biochemicals, Cleveland, Ohio) of the insert in this vector confirmed that the construct was correct, and that no undesired changes had been introduced.

The final coding sequence for TBP1(20-161)-hCGβ was assembled in a manner similar to that described for TBP1(20-161)-hCGα using fusion PCR and primers 1(αβ) and 4(β), and using as template the purified products from the p55 and hCG β fragments obtained from the first PCR reactions. The resulting pSVL plasmid containing the insert of interest was designated pSVLTBPhCGβ.

b. TBP(20-190)-hCG

A second set of TBP-hCG proteins was prepared by modification of the TBP(20-161)-hCG constructs to produce an analog containing TBP spanning from Asp20 to Thr190, in place of the 20-161 region in the initial analog. This was done by replacing the fragment between the BglII and XbaI sites in plasmid pSVLTBPhCGα with a PCR fragment containing the change. This PCR fragment was generated using fusion PCR. The primers were:

primer 1	TTT TAG ATC TCT TCT TGC ACA GTG GAC (SEQ ID NO:18)
primer 2	TGT GGT GCC TGA GTC CTC AGT (SEQ ID NO:19)
primer 3	ACT GAG GAC TCA GGC ACC ACA GCC GGT GCT GCC CCA GGT TG (SEQ ID NO:20)
primer 4	TTT TTC TAG AGA AGC AGC AGC AGC CCA TG (SEQ ID NO:21)

Primers 1 and 2 were used to generate the sequence coding the additional p55 residues from 161-190. The PCR reaction was performed essentially as described earlier, using 1 μg of each primer and pUC-p55 as template. Similarly, primers 3 and 4 were used to generate by PCR the linker between the 3'-end of the TBP-coding region, and the 5'-end of the hCG α subunit coding region, using as a template plasmid pSVLTBPhCGα. Products from these PCR reactions were confirmed

to be the correct size (about 296 bp and 121 bp respectively) by polyacrylamide gel electrophoresis (PAGE) on an 8% gel, and were then purified using a Wizard column. The design of primers 2 and 3 was such that they contained a region of overlap, so that the two PCR products (from primers 1 and 2, and from primers 3 and 4) could be annealed for fusion PCR with primers 1 and 4. Subsequent to the fusion reaction, the desired product of about 400 bp was confirmed and purified using a 1.5% agarose gel and a Wizard column. This DNA was then digested with BglII and XbaI, and ligated with BglII/XbaI-digested pSVLTBPhCGα. The presence of an insert in plasmids isolated from transformed AG1 *E. coli* was confirmed by digestion with BglII and XbaI. The new construct was designated pSVLTBP(20-190)-hCGα.

Similarly, plasmid pSVLTBPhCGβ was modified by substitution of the BglII-XcmI fragment. However, this was done by subcloning of a single PCR product, rather than with a fusion PCR product. Primers 1 and 2b (see below) were used with pUC-p55 as the template.

primer 2b TTT TCC ACA GCC AGG GTG GCA TTG ATG GGG
CGG CAC CGT GGA CCA GCA CCA GCT GTG GTG
CCT GAG TCC TCA GTG (SEQ ID NO:22)

The resulting PCR product (about 337bp) was confirmed and purified as described above, digested with BglII and XcmI, and then ligated into BglII/XbaI-digested pSVLTBPhCGβ. The presence of an insert in plasmids isolated from transformed AG1 *E. coli* was confirmed by digestion with BglII and XcmI. The new construct was designated pSVLTBP(20-190)-hCGβ.

The new constructs were subsequently confirmed by DNA sequencing.

In addition to producing these new pSVL-based plasmids, these constructs were also subcloned into other expression vectors likely to be more suitable for stable expression in CHO, particularly vector Dα, previously described as plasmid CLH3AXSV2DHFR (45). This was accomplished by converting a BamHI site flanking the inserts in the pSVL-based vectors to an XhoI site, and then excising the insert with XhoI and cloning it into XhoI digested Dα.

2. Transient and stable expression of the hybrid proteins

Transfections of COS-7 cells (ATCC CRL 1651, ref. 46) for transient expression of the TBP-hCG hybrid proteins were performed using electroporation (47). Exponentially growing COS-7 cells were removed by trypsinization, collected by gentle centrifugation (800 rpm, 4 minutes), washed with cold phosphate buffered saline (PBS), pH 7.3-7.4, and then repelleted by centrifugation. Cells were resuspended at a concentration of 5×10^6 cells per 400 μl cold PBS and mixed with 10 μg of plasmid DNA in a prechilled 2 mm gap electroporation cuvette. For cotransfections, 5 μg of each plasmid were used. The cuvette and cells were chilled on ice for a further 10 minutes, and then subjected to electroporation using a BTX Model 600 instrument and conditions of 125 V, 950μF and R=8. Afterward the cells were set to cool on ice for 10 minutes, transferred to a 15 ml conical tube containing 9.5 ml complete medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% L-glutamine) at room temperature, and left at room temperature for 5 minutes. After gentle mixing in the 15 ml tube, the entire contents was seeded onto two P100 plates and placed into a 37°C, 5% CO_2 incubator. After 18 hours the media was changed, and in some cases the new media contained only 1% or 0% FBS. After another 72 hours, the conditioned media was harvested, centrifuged to remove cells, and then stored frozen at -70°C.

Transfections of CHO-DUKX (CHO) cells for transient or stable expression were performed using calcium phosphate precipitation of DNA. Twenty-four hours prior to the transfection, exponentially growing CHO cells were plated onto 100 mm culture plates at a density of 7.5×10^5 cells per plate. On the day of the transfection, 10 μg of plasmid DNA was brought to 0.5 ml in transfection buffer (see below), 31 μl of 2 M $CaCl_2$ were added, the DNA-$CaCl_2$ solution was mixed by vortexing, and left to stand at room temperature for 45 minutes. After this the media was aspirated from the plates, the DNA was added to the cells using a sterile plastic pipette, and the cells were left at room temperature for 20 minutes. At

the end of this period, 5 ml of complete α(+)MEM containing 10% FBS was added to the plates, which were incubated at 37°C for 4-6 hours. The media was then aspirated off the plates, and the cells were subjected to a glycerol shock by incubating them with a solution of 15% glycerol in transfection buffer at 37°C for 3.5 minutes. After removal of the glycerol solution, the cells were washed twice with PBS, refed with 10 ml complete α(+)MEM, 10% FBS, and returned to the 37°C incubator. For stable transfections, after 48 hours the cells were split 1:10 and fed with selection medium (complete α-minus MEM (lacking nucleosides), 10% dialyzed FBS, and 0.02 μM methotrexate). Non-transfected (non-resistant) cells were typically eliminated in 3-4 weeks, leaving a population of transfected, methotrexate-resistant cells.

3. Quantitation of expression

Secretion of the hybrid proteins by transfected cells was assessed using a commercial assay kit for soluble p55 (R&D Systems; Minneapolis, Minnesota) in accordance with the manufacturer's instructions. This assay also provides an estimate of the hybrid protein levels in conditioned and processed media, which served as the basis for selecting doses to be used in the bioassay.

4. Assessment of heterodimer formation

To assess the ability of the TBP-hCG subunit fusions to combine and form heterodimers, a sandwich immunoassay using antibodies to the hCG subunits was performed. In this assay, a monoclonal antibody to the hCG β subunit is coated onto microtiter plates and used for analyte capture. The primary detection antibody is a goat polyclonal raised against the human TSH α subunit (#082422G - Biodesign International; Kennenbunkport, Maine), which is in turn detected using a horse radish peroxidase conjugated rabbit anti-goat polyclonal antibody (Cappel; Durham, North Carolina).

Several different anti-hCG β subunit antibodies were used in this work, all of which show no detectable cross-reactivity with the free α subunit. One of these antibodies (3/6) is used in the commercially available MAIAclone hCG assay kit (Biodata; Rome, Italy).

High-protein binding microtiter plates (Costar #3590) were coated with capture antibody by incubation (2 hours at 37°C) with 100 μl/well of a 5 μg/ml solution of antibody in coating buffer (PBS, pH 7.4, 0.1 mM Ca^{++}, 0.1 mM Mg^{++}). After washing once with wash solution (PBS, pH 7.4 + 0.1% Tween 20) the plate is blocked by completely filling the wells (~400 μl/well) with blocking solution (3% bovine serum albumin (BSA; fraction V - A-4503 Sigma) in PBS, pH 7.4) and incubating for one hour at 37°C or overnight at 4°C. The plate is then washed twice with wash solution, and the reference and experimental samples, diluted in diluent (5 mg/ml BSA in PBS, pH 7.4) to yield a 100 μl volume, are added. After incubating the samples and the plate for two hours at 37°C, the plate is again twice washed with wash solution. The primary detection antibody, diluted 1:5000 in diluent, is added (100 μl/well) and incubated for one hour at 37°C. The secondary detection antibody (HRP conjugated rabbit anti-goat Ig), diluted 1:5000 in diluent, is added (100 μl/well) and after incubation for one hour at 37°C, the plate is washed three times with wash solution. One hundred μl of TMB substrate solution (Kirkegaard and Perry Laboratories) is added, the plate is incubated 20 minutes in the dark at room temperature, and then the enzymatic reaction is stopped by addition of 50 μl/well 0.3M H_2SO_4. The plate is then analyzed using a microtiter plate reader set for a wavelength of 450 nm.

5. Partial purification

To better quantitate the activities of these hybrid proteins, TBP-hCG hybrid proteins were partially purified by immunoaffinity chromatography. The antibody used was a monoclonal commercially available from R&D Systems (MAB #225). The column was CNBr-activated sepharose, charged with the antibody by following the manufacturer's (Pharmacia) instructions.

Conditioned media was collected from confluent T-175 flasks of each line using daily harvests of 50 ml SFMII media (GIBCO), five harvests for each line. The collections were subjected to centrifugation (1000 RPM) to remove cellular debris. The material was then assayed for TBP content using

the commercial immunoassay and concentrated (Centricon units by Amicon; Beverly, Massachusetts) so that the apparent TBP concentration was about 50 ng/ml.

Ten ml of the concentrated TBP-hCG (sample #18873) was brought to approximately 1 M NaCl by addition of NaCl and adjustment of the solution to a conductivity of approximately 85 mS/cm. This was passed through a 0.5 ml anti-TBP immunoaffinity column. The flow-through was collected and run through the column a second time. After this the column was washed with 1 M NaCl in PBS. The bound TBP(20-161)-hCG was collected after elution with 50 mM citric acid (pH 2.5). The eluate (approximately 7 ml) was concentrated by filtration using Amicon Centricon-10's in accordance with the manufacturer's (Amicon) instructions, to a volume of approximately 200 μl. Approximately 800 μl of PBS was added to bring the sample volume to 1 ml, which was stored at 4°C until tested by bioassay.

6. Assessment of anti-TNF activity

Numerous *in vitro* TNF-induced cytotoxicity assays have been described for evaluating analogs of soluble TNF receptors. We utilized an assay employing a human breast carcinoma cell line, BT-20 cells (ATCC HTB 19). The use of these cells as the basis for a TNF bioassay has been described previously (48). These cells are cultured at 37°C in RPMI 1640 media supplemented with 10% heat-inactivated FBS. The cells were grown to a maximum 80-90% confluence, which entailed splitting every 3-4 days with a seeding density of about $3\text{x}10^6$ cells per T175cm^2 flask.

The BT-20 assay uses the inclusion of a cellular stain, crystal violet, as a detection method to assess survival of cells after treatment with TNF. Dead cells are unable to take up and retain the dye.

In brief, the protocol used for the assay of anti-TNF activity is the following. Recombinant human TNFα (R&D Systems) and the experimental samples are constituted in media (RPMI 1640 with 5% heat-inactivated FBS) and added to the wells of 96-well culture plates. The cells are then plated into these wells at a density of $1\text{x}10^5$ cells/well. The quantity of

TNFα added was determined earlier in titration studies, and represents a dose at which about 50% of the cells are killed.

After addition of the samples, the cells are cultured for 48 hours at 39°C, after which the proportion of live cells is determined using crystal violet staining and a microtiter plate reader (570 nm).

RESULTS

1. Constructs under study

The designs of the hybrid proteins studied are briefly summarized below; two control proteins, a monomeric soluble p55 (r-hTBP-1) and a dimeric TBP-immunoglobulin fusion protein (TBP-IgG3) (prepared essentially as described in (10)), were studied for comparative purposes.

Construct	TBP N-term	TBP C-term	Fusion partner
r-hTBP-1	mix of 9 and 20	180	none
TBP-IgG3	mix of 9 and 20	190	IgG3 heavy chain constant region
TBP(20-161)-hCG	20	161	hCGα and hCGβ (heterodimer)
TBP(20-190)-hCG	20	190	hCGα and hCGβ (heterodimer)

The sequences of the DNAs encoding, TBP(20-190)-hCG and TBP(20-161)-hCG are provided in Figures 1 and 2, respectively. A schematic summary of the constructs is provided in Figure 3.

2. Secretion of TBP-hCG proteins

All of the constructs tested were found to be produced and secreted into culture media by transfected mammalian cells. Data illustrating this are shown in Tables 1 and 2.

3. TBP-hCG(α/β) fusion proteins assemble into heterodimers

The combination of TBP-hCGα and TBP-hCGβ was confirmed using the sandwich assay for the hCG heterodimer.

Only the combined transfection of α and ß subunit fusions resulted in heterodimer detection (Table 3).

4. TBP-hCG hybrid proteins exhibit increased <u>activity over TBP monomer</u>

Hybrid proteins produced in either COS-7 or CHO cells were found to be potent inhibitors of TNFα in the BT-20 bioassay. Some of the samples tested are summarized in Table 4.

Negative controls (conditioned media from mock transfections) were included for the 1x media samples.

As illustrated in Figures 4-6 (points on y-axis), addition of TNF (2.5 ng/ml) results in a clear reduction in live cell number (as assessed by OD 570). In every case, active samples have as a maximal protective effect the restoration of cell viability to the level seen in the absence of added TNF (i.e., the control labeled "cells alone").

The positive controls, r-hTBP-1 and TBP-IgG3, are both protective, showing a clear dose-dependence and ED50s of approximately 100 ng/ml for the r-hTBP-1 (Figs. 4-6) and about 1.5 ng/ml for TBP-IgG3 (Fig. 4) respectively.

The TBP-hCG constructs from 1x media (CHO or COS) or from the immunopurification show dose-dependent protection, with approximate ED50s ranging from 2-11 ng/ml (Figs. 4-6).

The results from the *in vitro* bioassay are reported in Table 5. The data indicate that the hybrid proteins inhibit TNF cytotoxicity, and that they are substantially more potent than the TBP monomer. The negative controls were devoid of protective activity.

In addition to the possibility that dimerization of TBP may increase potency, it is also possible that the activity of the hybrid proteins are not related to dimeric interaction with TBP, but rather to steric inhibition due to the partner of the hybrid interfering with soluble TBP/TNF binding to cell-surface TNF receptors.

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference

herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional method steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

TABLES

Table 1: COS-7 transient expression (TBP ELISA)	
Hybrid Protein	Concentration (pg/ml)
TBP1	66
TBP-hCGα(20-161)	5.1
TBP-hCGβ(20-161)	0.5
TBP-hCG(20-161)	2.7
control	<0.25

Constructs were expressed using pSVL (Pharmacia)

Table 2: COS-7 transient expression (TBP ELISA)	
Hybrid protein	Concentration (ng/ml)
TBP1	131
TBP-hCGα(20-190)	81
TBP-hCGβ(20-190)	9
TBP-hCG(20-190)	62
control	<1

Constructs were expressed using a mouse metallothionein promoter-containing vector - pDα

Table 3: COS-7 transient expression (hCG heterodimer assay)	
Hybrid Protein	Concentration (ng/ml)
TBP1	<0.2
TBP-hCGα(20-190)	<0.2
TBP-hCGβ(20-190)	<0.2
TBP-hCG(20-190)	38
control	<0.2

Constructs were expressed using a mouse metallothionein promoter-containing vector - pDα

Table 4:Samples tested for anti-TNF activity		
Construct	*Cell source*	*Nature of sample*
r-hTBP-1	CHO	purified
TBP-IgG3	CHO	1x conditioned media
TBP(20-161)-hCG	CHO	immunopurified (anti-TBP)
TBP(20-190)-hCG	CHO	1x conditioned media
TBP(20-190)-hCG	COS	1x conditioned media

Table 5 :Preliminary Assessment of the hybrid proteins in TNF Cytotoxicity Assay

Construct	*Fusion partner*	*Anti-TNF activity (ED50) in BT-20 bioassay***
r-hTBP-1	none	100 ng/ml
TBP-IgG3	IgG3 heavy chain constant region	1.5 ng/ml
TBP(20-161)-hCG	hCGα and hCGβ (heterodimer)	2 ng/ml
TBP(20-190)-hCG	hCGα and hCGβ (heterodimer)	8-11 ng/ml

**The quantitation of material for dosing and estimation of ED50 was made using the TBP ELISA.

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1049 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 278..1047

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CTCACTATCG CCATGTAAGC CCAGTATTTG GCCAATCTCA GAAAGCTCCT CCTCCCTGGA     180

GGGATGGAGA GAGAAAAACA AACAGCTCCT GGAGCAGGGA GAGTGCTGGC CTCTTGCTCT     240

CCGGCTCCCT CTGTTGCCCT CTGGTTTCTC CCCAGGC TCC CGG ACG TCC CTG CTC     295
                                         Ser Arg Thr Ser Leu Leu
                                           1               5

CTG GCT TTT GGC CTG CTC TGC CTG CCC TGG CTT CAA GAG GGC AGT GCC      343
Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala
            10                  15                  20

GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCC      391
Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
        25                  30                  35

ATT TGC TGT ACC AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT      439
Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys
    40                  45                  50

CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC      487
Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser
 55                  60                  65                  70

TTC ACC GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC AAA      535
Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys
                75                  80                  85

TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC      583
Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp
            90                  95                 100

CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG      631
Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp
        105                 110                 115

AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT GGG      679
Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly
    120                 125                 130

ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC      727
Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys
135                 140                 145                 150

CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT GCC GGT      775
His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ala Gly
                155                 160                 165

GCT GCC CCA GGT TGC CCA GAA TGC ACG CTA CAG GAA AAC CCA TTC TTC      823
Ala Ala Pro Gly Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro Phe Phe
            170                 175                 180
```

```
TCC CAG CCG GGT GCC CCA ATA CTT CAG TGC ATG GGC TGC TGC TTC TCT        871
Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys Phe Ser
        185                 190                 195

AGA GCA TAT CCC ACT CCA CTA AGG TCC AAG AAG ACG ATG TTG GTC CAA        919
Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu Val Gln
    200                 205                 210

AAG AAC GTC ACC TCA GAG TCC ACT TGC TGT GTA GCT AAA TCA TAT AAC        967
Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser Tyr Asn
215                 220                 225                 230

AGG GTC ACA GTC ATG GGG GGT TTC AAA GTG GAG AAC CAC ACG GGG TGC       1015
Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr Gly Cys
                235                 240                 245

CAC TGC AGT ACT TGT TAT TAT CAC AAA TCT  TA AG                        1049
His Cys Ser Thr Cys Tyr Tyr His Lys Ser
            250                 255
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 256 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp
 1               5                  10                  15

Leu Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile
            20                  25                  30

His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr
        35                  40                  45

Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg
    50                  55                  60

Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu Arg His
65                  70                  75                  80

Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile
                85                  90                  95

Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn
            100                 105                 110

Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys
        115                 120                 125

Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln
    130                 135                 140

Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu
145                 150                 155                 160

Cys Val Ser Cys Ala Gly Ala Ala Pro Gly Cys Pro Glu Cys Thr Leu
                165                 170                 175
```

```
Gln Glu Asn Pro Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys
            180                 185                 190

Met Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys
        195                 200                 205

Lys Thr Met Leu Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys
    210                 215                 220

Val Ala Lys Ser Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val
225                 230                 235                 240

Glu Asn His Thr Gly Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser
                245                 250                 255
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1202 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 279..1199

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
CTCGAGATGG CTACAGGTAA GCGCCCCTAA AATCCCTTTG GGCACAATGT GTCCTGAGGG      60

GAGAGGTAGC GACCTGTAGA TGGGACGGGG GCACTAACCC TGAGGTTTGG GGCTTCTGAA     120

TGTGAGTATC GCCATGTAAG CCCAGTATTT GGCCAATGTC AGAAAGCTCC TGGTCCCTGG     180

AGGGATGGAG AGAGAAAAAC AAACAGCTCC TGGAGCAGGG AGAGTGCTGG CCTCTTGCTC     240

TCCGGCTCCC TCTGTTGCCC TGTGGTTTCT CCCCAGGC TCC CGG ACG TCC CTG         293
                                          Ser Arg Thr Ser Leu
                                                          260

CTC CTG GCT TTT GGC CTG CTC TGC CTG CCC TGG CTT CAA GAG GGC AGT       341
Leu Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp Leu Gln Glu Gly Ser
            265                 270                 275

GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT       389
Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn
        280                 285                 290

TCG ATT TGC TGT ACC AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC       437
Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp
    295                 300                 305

TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC       485
Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly
310                 315                 320                 325

TCT TTC ACC GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC       533
Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser
                330                 335                 340

AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG       581
Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val
            345                 350                 355
```

```
GAC CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT        629
Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr
        360                 365                 370

TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT        677
Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn
    375                 380                 385

GGG ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC        725
Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr
390                 395                 400                 405

TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT GCT        773
Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ala
                410                 415                 420

GGT GCT GGT CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG        821
Gly Ala Gly Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu
            425                 430                 435

AAG GAG GGC TGC CCC GTG TGC ATC ACC GTC AAC ACC ACC ATC TGT GCC        869
Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala
        440                 445                 450

GGC TAC TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTC CCC GCC        917
Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala
    455                 460                 465

CTG CCT CAG GTG GTG TGC AAC TAC CGC GAT GTG CGC TTC GAG TCC ATC        965
Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile
470                 475                 480                 485

CGG CTC CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCT       1013
Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala
                490                 495                 500

GTG GCT CTC AGC TGT CAA TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC       1061
Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp
            505                 510                 515

TGC GGG GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC       1109
Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe
        520                 525                 530

CAG GAC TCC TCT TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA       1157
Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro
    535                 540                 545

TCC CGA CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAA           1202
Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
550                 555                 560
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 307 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp
  1               5                  10                  15
```

```
Leu Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile
            20                  25                  30

His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr
        35                  40                  45

Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg
    50                  55                  60

Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu Arg His
65                  70                  75                  80

Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile
                85                  90                  95

Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn
            100                 105                 110

Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys
        115                 120                 125

Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln
    130                 135                 140

Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu
145                 150                 155                 160

Cys Val Ser Cys Ala Gly Ala Gly Pro Arg Cys Arg Pro Ile Asn Ala
                165                 170                 175

Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn
            180                 185                 190

Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln
        195                 200                 205

Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val
    210                 215                 220

Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro
225                 230                 235                 240

Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg
                245                 250                 255

Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys
            260                 265                 270

Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro
        275                 280                 285

Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile
    290                 295                 300

Leu Pro Gln
305
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1147 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

```
(ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 278..1132

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCGAGATGGC TACAGGTAAG CGCCCCTAAA ATCCCTTTGG GCACAATGTG TCCTGAGGGG      60

AGAGGCAGCG ACCTGTAGAT GGGACGGGGG CACTAACCCT CAGGTTTGGG GCTTTTGAAT     120

GTGAGTATGG CCATGTAAGC CCAGTATTTG CCCAATCTCA GAAAGCTCCT GGTCCCTGGA     180

GGGATGGAGA GAGAAAAACA AACAGCTCCT GGAGCAGGGA CACTCCTGGC CTCTTGCTCT     240

GCGGCTCCGT GTGTTGCCCT GTGGTTTCTC CCCACGC TCC CGG ACG TCC CTG CTC      295
                                         Ser Arg Thr Ser Leu Leu
                                                     310

CTG GCT TTT GGC CTG CTC TGC CTG CCC TGG CTT CAA GAG GGC AGT GCC      343
Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala
    315                 320                 325

GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG      391
Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
330                 335                 340                 345

ATT TGC TGT ACC AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT      439
Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys
                350                 355                 360

CCA GGC CCG GGG CAG GAT ACC GAC TGC AGG GAG TGT GAG AGC GGC TCC      487
Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser
            365                 370                 375

TTC ACC GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC AAA      535
Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys
        380                 385                 390

TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC      583
Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp
    395                 400                 405

CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG      631
Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp
410                 415                 420                 425

AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC ACC CTC TGC CTC AAT GGG      679
Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Thr Leu Cys Leu Asn Gly
                430                 435                 440

ACC GTG CAC CTC TCC TGT CAG GAG AAA CAG AAC ACC GTC TGC ACC TGC      727
Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys
            445                 450                 455

CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT AGT AAC      775
His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn
        460                 465                 470

TGT AAG AAA AGC CTG GAG TGC ACG AAG TTG TCC CTA CCC CAG ATT GAG      823
Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Ser Leu Pro Gln Ile Glu
    475                 480                 485

AAT GTT AAG GGC ACT GAG GAC TCA GGC ACC ACA GCC GGT GCT GCC CCA      871
Asn Val Lys Gly Thr Glu Asp Ser Gly Thr Thr Ala Gly Ala Ala Pro
490                 495                 500                 505
```

```
GGT TGC CCA GAA TGC ACG CTA CAG GAA AAC CCA TTC TTC TCC CAG CCG        919
Gly Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro Phe Phe Ser Gln Pro
                510                 515                 520

GGT GCC CCA ATA CTT CAG TGC ATG GGC TGC TGC TTC TCT AGA GCA TAT        967
Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys Phe Ser Arg Ala Tyr
            525                 530                 535

CCC ACT CCA CTA AGG TCC AAG AAG ACG ATG TTG GTC CAA AAG AAC GTC       1015
Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu Val Gln Lys Asn Val
        540                 545                 550

ACC TCA GAG TCC ACT TGC TGT GTA GCT AAA TCA TAT AAC AGG GTC ACA       1063
Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser Tyr Asn Arg Val Thr
    555                 560                 565

GTA ATG GGG GGT TTC AAA GTG GAG AAC CAC ACG GCG TGC CAC TGC AGT       1111
Val Met Gly Gly Phe Lys Val Glu Asn His Thr Ala Cys His Cys Ser
570                 575                 580                 585

ACT TGT TAT TAT CAC AAA TCT TAAGGATCCC TCGAG                          1147
Thr Cys Tyr Tyr His Lys Ser
                590
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp
  1               5                  10                  15

Leu Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile
             20                  25                  30

His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr
         35                  40                  45

Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg
     50                  55                  60

Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu Arg His
 65                  70                  75                  80

Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile
                 85                  90                  95

Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn
            100                 105                 110

Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys
        115                 120                 125

Thr Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln
    130                 135                 140

Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu
145                 150                 155                 160

Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu
                165                 170                 175
```

```
Ser Leu Pro Gln Il  Glu Asn Val Lys Gly Thr Glu Asp Ser Gly Thr
            180                 185                 190

Thr Ala Gly Ala Ala Pro Gly Cys Pro Glu Cys Thr Leu Gln Glu Asn
        195                 200                 205

Pro Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys
    210                 215                 220

Cys Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met
225                 230                 235                     240

Leu Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys
                245                 250                 255

Ser Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His
            260                 265                 270

Thr Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser
        275                 280                 285
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1301 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 279..1287

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CTCGAGATGG CTACAGGTAA GCGCCCCTAA AATCCCTTTG GGCACAATGT GTCCTGAGGG      60

GAGAGGCAGC GACCTGTAGA TGGGACGGGG GCACTAACCC TCAGGTTTGG GGCTTCTGAA     120

TGTGAGTATC GCCATGTAAG CCCAGTATTT GGCCAATGTC AGAAAGCTCC TGGTCCCTGG     180

AGGGATGGAG AGAGAAAAAC AAACACCTCC TGGAGCAGGG AGAGTGCTGC CCTCTTGCTC     240

TCCGGCTCCC TCTGTTGCCC TCTGGTTTCT CCCCAGGC TCC CGG ACG TCC CTG         293
                                          Ser Arg Thr Ser Leu
                                                          290

CTC CTG GCT TTT GGC CTG CTC TGC CTG CCC TGG CTT CAA GAG GGC AGT      341
Leu Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp Leu Gln Glu Gly Ser
                295                 300                 305

GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT      389
Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn
            310                 315                 320

TCG ATT TGC TGT ACC AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC      437
Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp
        325                 330                 335

TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC      485
Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly
    340                 345                 350
```

```
TCC TTC ACC GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC        533
Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser
355                 360                 365                 370

AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG        581
Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val
                375                 380                 385

GAC CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT        629
Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr
            390                 395                 400

TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT        677
Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn
        405                 410                 415

GGG ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC        725
Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr
    420                 425                 430

TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT AGT        773
Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser
435                 440                 445                 450

AAC TGT AAG AAA AGC CTG GAG TGC ACG AAG TTG TGC CTA CCC CAG ATT        821
Asn Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile
                455                 460                 465

GAG AAT GTT AAG GGC ACT GAG GAC TCA GGC ACC ACA GCT GGT GCT GGT        869
Glu Asn Val Lys Gly Thr Glu Asp Ser Gly Thr Thr Ala Gly Ala Gly
            470                 475                 480

CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG GGC        917
Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly
        485                 490                 495

TGC CCC GTG TGC ATC ACC GTC AAC ACC ACC ATC TGT GCC GGC TAC TGC        965
Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys
    500                 505                 510

CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT CAG       1013
Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
515                 520                 525                 530

GTG GTG TGC AAC TAC CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC CCT       1061
Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu Pro
                535                 540                 545

GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT CTC       1109
Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala Leu
            550                 555                 560

AGC TGT CAA TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG GGT       1157
Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly
        565                 570                 575

CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC TCC       1205
Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser
    580                 585                 590

TCT TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA CTC       1253
Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu
595                 600                 605                 610

CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA T AAGGATCCCT CGAG         1301
Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
                615                 620
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 336 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp
 1               5                  10                  15

Leu Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile
            20                  25                  30

His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr
        35                  40                  45

Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg
    50                  55                  60

Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu Arg His
65                  70                  75                  80

Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile
                85                  90                  95

Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn
            100                 105                 110

Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys
        115                 120                 125

Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln
    130                 135                 140

Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu
145                 150                 155                 160

Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu
                165                 170                 175

Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser Gly Thr
            180                 185                 190

Thr Ala Gly Ala Gly Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala
        195                 200                 205

Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile
    210                 215                 220

Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu
225                 230                 235                 240

Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu
                245                 250                 255

Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser
            260                 265                 270

Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser Thr
        275                 280                 285
```

```
Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro
    290                 295                 300

Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro
305                 310                 315                 320

Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
                325                 330                 335
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Ala Gly Ala Ala Pro Gly
1               5
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Ala Gly Ala Gly
1
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTTTCTCGAG ATGGCTACAG GTAAGCGCCC 30

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACCTGGGGCA GCACCGGCAC AGGAGACACA CTCGTTTTC 39

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGTGCCGGTG CTGCCCCAGG TTGCCCAGAA TGCACGCTAC AG 42

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTTTGGATCC TTAAGATTTG TGATAATAAC AAGTAC 36

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCGTGGACCA GCACCAGCAC AGGAGACACA CTCGTTTTC 39

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGTGCTGGTG CTGGTCCACG GTGCCGCCCC ATCAAT 36

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TTTTGGATCC TTATTGTGGG AGGATCGGGG TG 32

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTTTAGATCT CTTCTTGCAC AGTGGAC 27

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGTGGTGCCT GAGTCCTCAG T 21

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ACTGAGGACT CAGGCACCAC AGCCGGTGCT GCCCCAGGTT G 41

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

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(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TTTTCCACAG CCAGGGTGGC ATTGATGGGG CGGCACCGTG GACCAGCACC AGCTGTGGTG 60

CCTGAGTCCT CAGTG 75

CLAIMS

1. A hybrid protein comprising two coexpressed amino acid sequences forming a dimer, each comprising:

a) at least one amino acid sequence selected from the group consisting of a homomeric receptor, a chain of a heteromeric receptor, a ligand, and fragments thereof which retain the ligand-receptor binding capability; and

b) a subunit of a heterodimeric proteinaceous hormone, or fragments thereof which retain the ability of the subunit to form a heterodimer with other subunits thereof;

wherein sequences (a) and (b) are bonded directly or through a peptide linker, and in which the sequence (b) in each of said two coexpressed sequences are capable of aggregating to form a dimer complex.

2. A hybrid protein in accordance with claim 1, wherein said sequence (a) is selected from the group consisting of TBP1, TBP2 or fragments thereof still containing the ligand binding domain; the extracellular domain of the IFNα/β receptor or the IFNγ receptor; a gonadotropin receptor or extracellular fragments thereof; antibody light chains or fragments thereof, optionally associated with the respective heavy chains; antibody heavy chains or fragments thereof; antibody Fab domains; and IL-6, IFN-β, TPO or fragments thereof.

3. A hybrid protein in accordance with claim 1, wherein said sequence (b) is selected from the group consisting of subunits of hCG, FSH, LH, TSH or inhibin, and fragments thereof.

4. A hybrid protein in accordance with claim 1, wherein sequence (a) is linked to the amino terminus of sequence (b).

5. A hybrid protein in accordance with claim 1, wherein sequence (a) is linked to the carboxy terminus of sequence (b).

6. A hybrid protein in accordance with claim 1, wherein said two coexpressed amino acid sequences each include the sequence for TBP1 or the fragment thereof corresponding to amino acid residues 20-161 or 20-190 of TBP1, as sequence (a) and the respective α and β subunits of hCG or fragments thereof, as sequence (b).

7. A hybrid protein in accordance with claim 1, wherein said two coexpressed amino acid sequences each include the extracellular domain of a gonadotropin receptor as sequence (a) and the respective α and β subunits of a gonadotropin as sequence (b).

8. A hybrid protein in accordance with claim 7, wherein said sequence (a) is the FSH receptor extracellular domain and sequence (b) is a subunit of FSH.

9. A hybrid protein in accordance with claim 7, wherein said sequences (a) and (b) are linked with a peptide linker.

10. A hybrid protein in accordance with claim 9, wherein said peptide linker has an enzyme cleavage site.

11. A hybrid protein in accordance with claim 10, wherein said enzyme cleavage site is a thrombin cleavage site.

12. A hybrid protein in accordance with claim 10, wherein said enzyme cleavage site is recognized and cleaved by an enzyme which is found in the ovary.

13. A hybrid protein in accordance with claim 9, wherein said peptide linker serves as a flexible hinge.

14. A hybrid protein in accordance with claim 1, wherein one or more covalent bonds between the two subunits (b) are added.

15. A DNA molecule encoding a hybrid protein in accordance with claim 1.

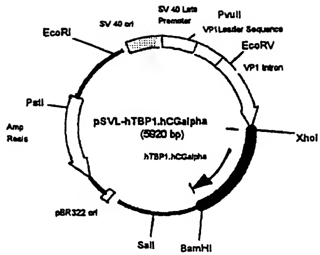
16. An expression vector containing a DNA molecule in accordance with claim 15.

17. A host cell containing an expression vector in accordance with claim 16 and capable of expressing said hybrid protein.

18. A method for producing hybrid protein comprising culturing a host cell in accordance with claim 17 and recovering the hybrid protein expressed thereby.

19. A pharmaceutical composition comprising a hybrid protein in accordance with claim 1 and a pharmaceutically acceptable carrier and/or excipient.

20. A method for inducing follicular maturation, comprising administering a pharmaceutical composition comprising the hybrid protein of claim 8 to a subject in need thereof.



Xho I hGH Signal Sequence hGH Intron

TCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAGAGGCAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGGGCTTCT
Met Ala Thr

GAATGTGAGTATCGCCATGTAAGCCCAGTATTTGGCCAATCTCAGAAAGCTCCTGGTCCCTGGAGGGATGGAGAGAGAAAAACAAACAGCTCCTGGAGCAGGGAGAGTGCTGGCCTCTTGCTCT

CGGCTCCCTCTGTTGCCCTCTGGTTTCTCCCCAGGC TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC TGC CTG CCC TGG CTT
Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp Leu

+20 Asp of Processed TBP1

CAA GAG GGC AGT GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG ATT TGC TGT ACC AAG TGC CAC AAA GGA
Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly

ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC GCT TCA GAA AAC CAC CTC
Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu

AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC CGG GAC ACC GTG TGT GGC TGC
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys

AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT GGG ACC GTG CAC CTC TCC TGC
Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys

Linker

CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT GCC GGT GCT GCC CCA GGT
Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ala Gly Ala Ala Pro Gly

+7 Cys of hCG alpha

TGC CCA GAA TGC ACG CTA CAG GAA AAC CCA TTC TTC TCC CAG CCG GGT GCC CCA ATA CTT CAG TGC ATG GGC TGC TGC TTC TCT AGA GCA TAT
Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys Phe Ser Arg Ala Tyr

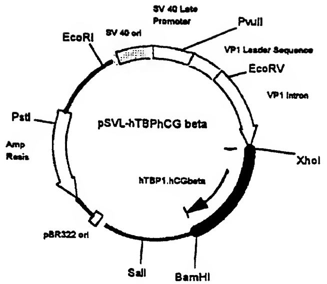
CCC ACT CCA CTA AGG TCC AAG AAG ACG ATG TTG GTC CAA AAG AAC GTC ACC TCA GAG TCC ACT TGC TGT GTA GCT AAA TCA TAT AAC AGG GTC
Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser Tyr Asn Arg Val

ACA GTA ATG GGG GGT TTC AAA GTG GAG AAC CAC ACG GCG TGC CAC TGC AGT ACT TGT TAT TAT CAC AAA TCT TAA G
Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser ···

Bam HI

Figure 1 (a)

TBP(20-161)-hCGα FUSION CONSTRUCT



XhoI hGH Signal Sequence hGH Intron

CTCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAGAGGCAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGG
Met Ala Thr

GCTTCTGAATGTGAGTATCGCCATGTAAGCCCAGTATTTGGCCAATCTCAGAAAGCTCCTGGTCCCTGGAGGGATGGAGAGAGAAAAACAAACAGCTCCTGGAGCAGGGAGAGTGCTGGC

CTCTTGCTCTCCGGCTCCCTCTGTTGCCCTCTGGTTTCTCCCCAGGC TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC TGC CTG
Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu
+20 Asp of Processed TBP1

CCC TGG CTT CAA GAG GGC AGT GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG ATT TGC TGT ACC
Pro Trp Leu Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr

AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC
Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr

GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC
Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp

CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC
Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu

AAT GGG ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC
Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val
Linker +7 Pro of hCG beta

TCC TGT *GCT GGT GCT GGT* CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG GGC TGC CCC GTG TGC ATC ACC GTC
Ser Cys Ala Gly Ala Gly Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val

AAC ACC ACC ATC TGT GCC GGC TAC TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT CAG GTG GTG TGC AAC TAC
Asn Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr

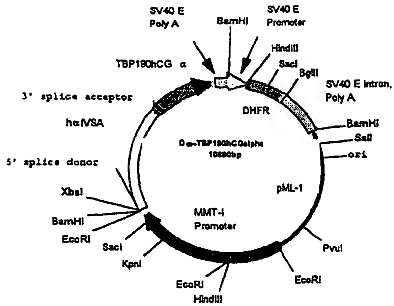
CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT CTC AGC TGT CAA
Arg Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln

TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC TCC TCT
Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser

TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAA
Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln ••• Bam HI

Figure 1 (b)

TBP(20-161)-hCGβ FUSION CONSTRUCT



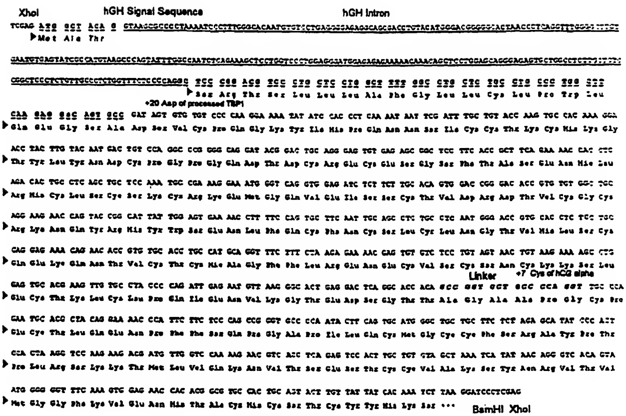
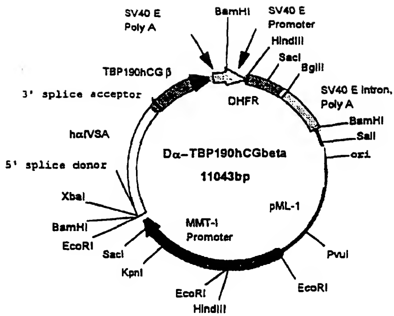


Figure 2(a)
TBP(20-190)-hCGα FUSION CONSTRUCT



XhoI hGH Signal Sequence hGH Intron

CTCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAGAGGCAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGG
Met Ala Thr

GCTTCTGAATGTGAGTATCGCCATGTAAGCCCAGTATTTGGCCAATCTCAGAAAGCTCCTGGTCCCTGGAGGGATGGAGAGAGAAAAACAAACAGCTCCTGGAGCAGGGAGAGTGCTGGC

CTCTTGCTCTCCGGCTCCCTCTGTTGCCCTCTGGTTTCTCCCCAGGC TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC TGC CTG
Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu

+20 Asp of Processed TBP1

CCC TGG CTT CAA GAG GGC AGT GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG ATT TGC TGT ACC
Pro Trp Leu Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr

AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC
Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr

GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC
Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp

CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC
Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu

AAT GGG ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC
Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val

TCC TGT AGT AAC TGT AAG AAA AGC CTG GAG TGC ACG AAG TTG TGC CTA CCC CAG ATT GAG AAT GTT AAG GGC ACT GAG GAC TCA GGC ACC
Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser Gly Thr

Linker +7 Pro of beta

ACA GCT GGT GCT GGT CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG GGC TGC CCC GTG TGC ATC ACC GTC AAC
Thr Ala Gly Ala Gly Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn

ACC ACC ATC TGT GCC GGC TAC TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT CAG GTG GTG TGC AAC TAC CGC
Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg

GAT GTG CGC TTC GAG TCC ATC CGG CTC CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT CTC AGC TGT CAA TGT
Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys

GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC TCC TCT TCC
Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser

TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAA GGATCCCTCGAG
Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln ··· BamHI XhoI

Figure 2 (b)
TBP(20-190)-hCGβ FUSION CONSTRUCT

Figure 4. CHO cell expressed TBP-hCG(20-190) inhibits TNFα-induced cytotoxicity on BT-20 cells

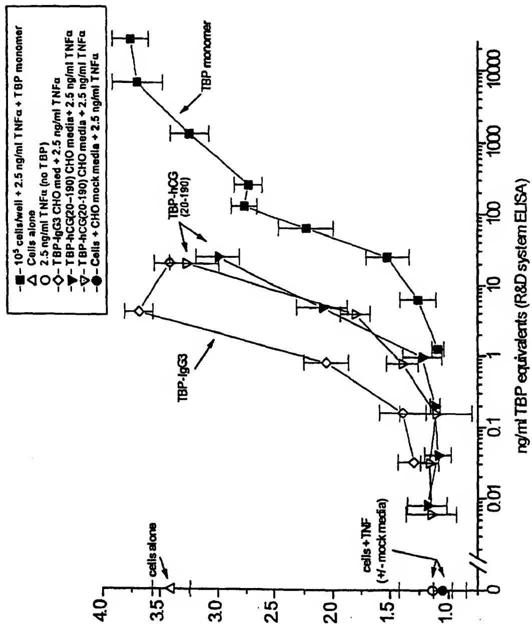


Figure 5. COS cell expressed TBP-hCG(20-190) inhibits TNFα-induced cytotoxicity on BT-20 cells

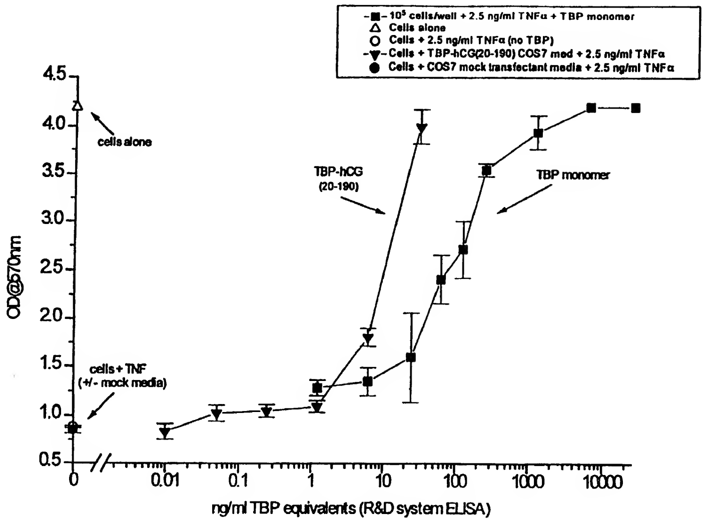
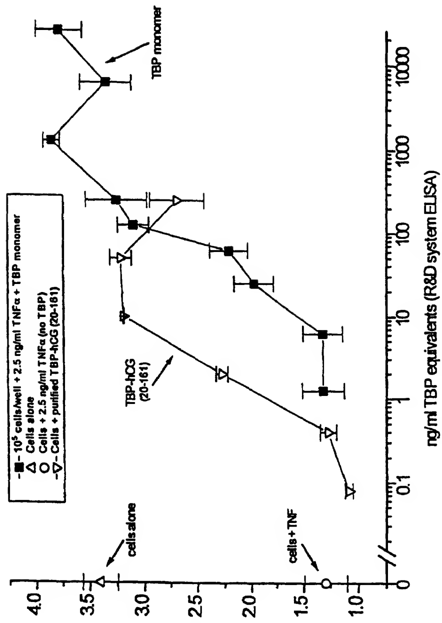


Figure 6. Affinity purified CHO cell expressed TBP-hCG(20-161) inhibits TNFα-induced cytotoxicity on BT-20 cells



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/02315

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/62 C12N15/16 C07K14/59 C07K14/715 C07K14/72
C07K16/46 C12N15/85 C12N5/10 A61K38/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 31544 A (YEDA RESEARCH AND DEVELOPMENT CO. LTD.) 23 November 1995 cited in the application see page 6, line 28 - line 39 see page 7, line 4 - page 11, line 12 ---	1-20
A	MOL. ENDOCRINOL. (1995), 9(12), 1720-6 CODEN: MOENEN;ISSN: 0888-8809, 1995, XP000675344 NARAYAN, PREMA ET AL: "Functional expression of yoked human chorionic gonadotropin in baculovirus-infected insect cells" see abstract see page 1721, right-hand column, paragraph 2 - page 1724, left-hand column, paragraph 1 --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
10 June 1997	01.07.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/US 97/02315

C.(Continuation) DOCUMENTS C NSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOLOGY OF REPRODUCTION, vol. 52, no. 1, January 1995, pages 68-73, XP000675391 GREGORY A. JOHNSON ET AL.: "Baculovirus-Insect cell production of bioactive Choriogonadotropin-Immunoglobulin G heavy-chain fusion proteins in sheep" cited in the application see the whole document ---	1-20
P,X	J. BIOL. CHEM. (1996), 271(49), 31638-31642 CODEN: JBCHA3;ISSN: 0021-9258, 1996, XP002032680 WU, CHENGBIN ET AL: "Protein engineering of a novel constitutively active hormone-receptor complex" see abstract see page 31638, right-hand column, paragraph 2 - paragraph 3 see page 31639, left-hand column, paragraph 4 - right-hand column, paragraph 2 see page 31640, right-hand column, paragraph 4 - page 31641, right-hand column, paragraph 4 -----	1-5,7,9, 10,13-18

1

INTERNATIONAL SEARCH REPORT	International application No. PCT/US 97/02315

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 20
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 20 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/02315

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9531544 A	23-11-95	AU 2546995 A	05-12-95
		CA 2189983 A	23-11-95
		EP 0759984 A	05-03-97
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